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16. ABSTRACT

To assess possible maternal hepatic and reproductive effects of this uncharged, low molecular weight, lipophilic chlorinated benzene 0, 100, 300 and 1000 mg/kg/day of 1,2,3,4-tetrachlorobenzene (TCB) was orally administered to pregnant rats on days 9-13 of gestation and the animals were killed on day 14 of pregnancy. Phenobarbital and β-naphthoflavone were administered to other pregnant rats as positive hepatic controls. Maternal mortality (7/19 rats) was increased and body weight gain was greatly decreased in the 1000 mg/kg/day TCB group. Liver to body weight ratio and hepatic microsomal protein content were unaffected by any TCB treatment. On day 14 maternal NADPH-cytochrome c reductase activity was increased at 1000 mg/kg/day, while the maternal hepatic microsomal cytochrome P-450 content was significantly induced by both 300 and 1000 mg/kg/day of TCB. Microsomal N-demethylation of aminopyrine was increased from 2.6 to 4.0 and 4.5 nmol/mg protein/min at doses of 300 and 1000 mg/kg TCB, respectively. However, maternal hepatic microsomal ethoxyresorufin O-deethylase activity was not consistently increased by TCB. Hepatic glutathione S-transferase activity towards 1,2-dichloro-4-nitrobenzene was increased only by the 1000 mg/kg/day TCB treatment. The rate of microsomal p-nitrophenol and phenolphthalein glucuronidation was increased by TCB administration.

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MATERNAL HEPATIC AND EMBRYONIC EFFECTS OF 1,2,3,4-TETRACHLOROBENZENE IN THE RAT*

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SUMMARY

To assess possible maternal hepatic and reproductive effects of this uncharged, low molecular weight, lipophilic chlorinated benzene 0, 100, 300 and 1000 mg/kg/day of 1,2,3,4-tetrachlorobenzene (TCB) was orally administered to pregnant rats on days 9—13 of gestation and the animals were killed on day 14 of pregnancy. Phenobarbital and β-naphthoflavone were administered to other pregnant rats as positive hepatic controls. Maternal mortality (7/19 rats) was increased and body weight gain was greatly decreased in the 1000 mg/kg/day TCB group. Liver to body weight ratio and hepatic microsomal protein content were unaffected by any TCB treatment. On day 14 maternal NADPH-cytochrome *c* reductase activity was increased at 1000 mg/kg/day, while the maternal hepatic microsomal cytochrome *P*-450 content was significantly induced by both 300 and 1000 mg/kg/day of TCB. Microsomal N-demethylation of aminopyrine was increased from 2.6 to 4.0 and 4.5 nmol/mg protein/min at doses of 300 and 1000 mg/kg TCB, respectively. However, maternal hepatic microsomal ethoxyresorufin *O*-deethylase activity was not consistently increased by TCB. Hepatic glutathione *S*-transferase activity towards 1,2-dichloro-4-nitrobenzene was increased only by the 1000 mg/kg/day TCB treatment. The rate of microsomal *p*-nitrophenol and phenolphthalein glucuronidation was increased

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Abbreviation: TCB, tetrachlorobenzene.

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by TCB administration. Embryonic growth was adversely affected by TCB treatment. Yolk sac diameter, embryonic crown-rump length, and head length were all decreased by treatment with 300 mg/kg/day TCB. This TCB treatment did not significantly elevate the number of dead or abnormal embryos.

Key words: 1,2,3,4-Tetrachlorobenzene; Chlorinated benzenes; Hepatic; Teratogenicity; Cytochrome *P*-450; UDP-glucuronyltransferase; Glutathione *S*-transferase

INTRODUCTION

Chlorinated benzenes are industrial compounds which are present in the environment. The number and position of chlorine atoms on the benzene ring influences the lipid solubility, biotransformation and toxicity of the molecule [1- 3]. Chlorinated benzenes have low molecular weight and lack of charge which should permit substantial placental transport of these lipophilic compounds. Because chlorinated benzenes have been found in leaking chemical waste dumps, concern about their possible toxic, reproductive and teratogenic health effects has arisen.

1,2,3,4-Tetrachlorobenzene (TCB) was chosen for study as it is the most highly chlorinated benzene which still possesses the 2 vicinal hydrogens required for epoxidation and activation [4] to possible embryotoxic metabolites. Previous work with 1,2,4,5-TCB, which lacks 2 vicinal hydrogens, showed no embryo toxicity, teratogenicity or growth retardation [5]. 1,2,3,4-TCB should form reactive epoxides and deplete maternal glutathione [2]. Thus, studies with 1,2,3,4-TCB could provide insight into the reactive intermediate theory of embryonic toxicity and teratogenicity.

Reactive intermediates have recently been directly implicated as a mechanism of teratogenesis by investigations in a coupled microsomal activating/embryo culture system [6]. Some in vivo teratogenesis might be caused by hepatic formation of long lived intermediates (e.g., 4-hydroxycyclophosphamide) [7] and transport of these intermediates to the developing conceptus. Alternatively, reactive intermediates might be formed in embryonic or fetal tissues even though the rates of formation in these tissues are substantially less than that of the maternal animal [8,9]. Using day 7.5 mouse embryos, Galloway et al. [10] observed increases in the sister chromatid exchange frequency in benzo[α]pyrene treated embryos. This effect was interpreted to be due to a low rate of benzo[α]pyrene activation in the embryonic tissue. Filler and Lew [10] cultured preimplantation mouse embryos and detected benzo[α]pyrene metabolism in vitro and also demonstrated embryonic enzyme induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in day 3.5 blastocysts. In inbred mouse strains exposed to 2,3,7,8-

tetrachlorodibenzo-*p*-dioxin, a high percentage of cleft palate (54–95%) was found in those strains which possessed a high affinity Ah induction receptor [12]. In inbred mice with a lower affinity Ah induction receptor, a lower rate of cleft palate (0–3%) following exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was observed [12]. Although rapid epoxidation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin does not occur in the rat, the metabolites 1-hydroxy-2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 8-hydroxy-2,3,7-trichlorodibenzo-*p*-dioxin have been identifed after incubation with isolated rat hepatocytes [13]. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin is an extremely potent hepatic cytochrome *P*-448 inducer [14] which greatly increases the epoxidation rate of some environmental and endogenous compounds.

In the present study the maternal hepatic and embryonic effects of administration of 100, 300, and 1000 mg/kg/day of 1,2,3,4-tetrachlorobenzene were determined. TCB was administered on days 9–13 of pregnancy and experimental animals killed on the 14th day. Because the response of liver from pregnant rats to xenobiotics is not as well characterized as is the response in males or non-pregnant females, phenobarbital and β-naphthoflavone treatment groups were included in this study for comparative purposes. Day 14 embryos exposed to TCB in vivo were evaluated for growth and morphological parameters. The gestational chemical exposure period used in these studies is part of the period of susceptibility to teratogenesis in the rat. Embryos were evaluated on the day 14 of gestation because present in vitro post implantation embryo culture methods are limited to days 9–14 of gestation [15].

METHODS

Experimental animals and dosing

Time pregnant Sprague–Dawley rats (CD strain, 220—270 g) were obtained from Charles River (Kingston, NY) and given food and water ad libitum. The day sperm were detected in the vaginal smear is referred to as day 1 of gestation. Unpurified 1,2,3,4-TCB (Aldrich Chem. Co., Milwaukee, WI > 98% pure) was ground to a fine powder with a mortar and pestle and suspended in 1.5% gum tragacanth (Sigma Chem. Co., St. Louis, MI). Control animals were given 1.5% gum tragacanth. The suspensions were orally administered to pregnant rats on days 9–13 of gestation in a volume of 2 ml/kg. Sodium phenobarbital (Sigma Chem. Co.) and β-naphthoflavone (Aldrich Chem. Co.) were dissolved in 0.9% saline and corn oil, respectively. They were given intraperitoneally (1 ml/kg) in doses of 100 mg/kg/day and 80 mg/kg/day, respectively. Animals were killed by decapitation in the morning of day 14 and their livers excised and weighed. Hematoxylin and eosin stained sections of formalin fixed control and treated livers were examined histologically by standard procedures. Histological evaluations were preformed on all rat livers from which biochemical parameters were determined. Uteri were removed and the embryos examined for growth and differentiation parameters as described previously [6]. Day 14 live

embryos were observed with a Wild 7A stereomicroscope and scored as normal or abnormal as compared to gum tragacanth treated controls.

Enzyme assays

Five grams of maternal rat liver was homogenized in 20 ml of 0.02 M Tris—HCl buffer (pH 7.5) containing 1.15 M KCl. Washed microsomes were prepared by ultracentrifugation as described by Kitchin and Woods [16]. Cytochrome *P*-450 content was assayed by the method of Omura and Sato [17] using an Aminco DW-2 spectrophotometer. NADPH-cytochrome *c* reductase [18], aminopyrine *N*-demethylase [19,20], ethoxyresorufin *O*-deethylase [21] and glutathione *S*-transferase [22] activities

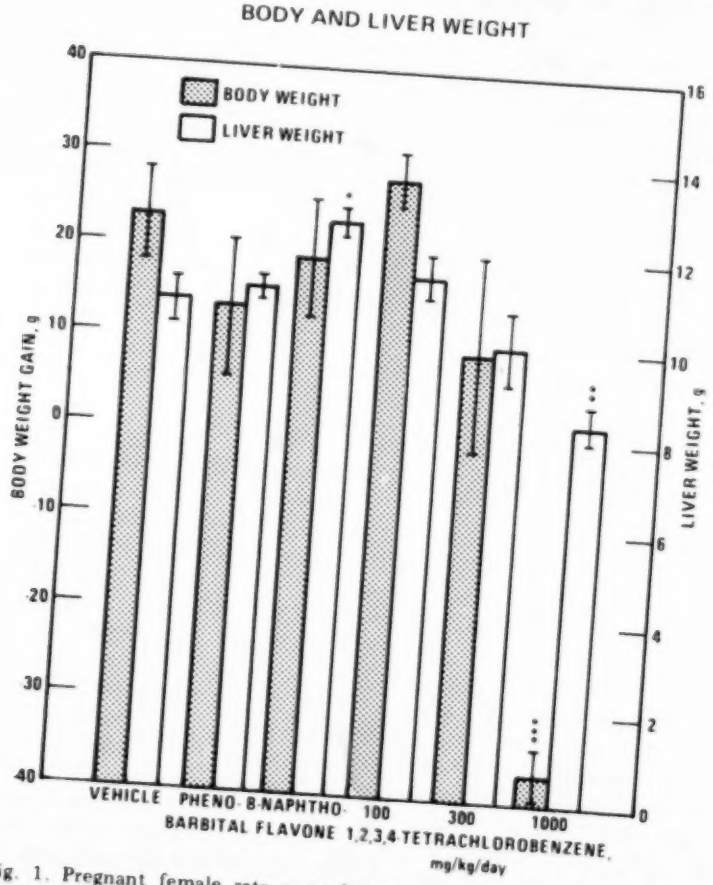

Fig. 1. Pregnant female rats were fed the indicated compound during days 9—13 of gestation. The body weight gain (gestational days 14—8) and day 14 liver weights are expressed as the mean + S.E.M. for 5—7 animals. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

were determined by published procedures. Hepatic reduced glutathione content was determined by the method of Sedlak and Lindsay [23]. The in vitro rates of the glucuronidation of *p*-nitrophenol (0.8 mM substrate) and phenolphthalein (0.2 mM substrate) in the presence of 1.4 mM UDP-glucuronic acid were determined by substrate disappearance using 0.2% Triton X-100 activated microsomes [24]. The microsomal protein content was determined by the biuret method [25].

Statistics

Analysis of variance procedures were used to determine statistically significant effects [26]. The William's test [27] was used to determine the minimal dose-related effects while enumerative data was analyzed by the Fishers exact test [26]. Quantitative data is presented as the mean ± S.E.M. for 5 or more independent observations. Twelve and 15 litters were used to determine the embryonic effects of gum tragacanth or TCB administration. In interpreting the embryonic data the litter was considered the statistical unit.

RESULTS

Maternal lethality occurred only in the phenobarbital (1/8 rats), 300 mg/kg/day TCB (1/10) and the 1000 mg/kg/day TCB (7/19) treatment groups. The significant mortality at 1000 mg/kg of TCB was accompanied by a drop in body weight (−37 grams) and liver weight (Fig. 1). Liver weight was increased by β-naphthoflavone but not by phenobarbital administration while the maternal hepatic microsomal protein content was unaffected by any compound tested.

Maternal hepatic cytochrome *P*-450 content was increased from 0.73 to 1.21 and 1.72 nmol/mg protein in the 300 and 1000 mg/kg/day TCB treatment groups (Fig. 2). NADPH-cytochrome *c* reductase activity was increased from 116 to 161 nmol/mg protein/min at the highest dose of TCB used. β-Naphthoflavone increased total hepatic cytochrome *P*-450 level more than did phenobarbital.

In contrast, aminopyrine *N*-demethylase activity (a cytochrome *P*-450 type substrate [28]) was induced by phenobarbital (2.2-fold), 300 mg/kg/day TCB (1.5-fold) and 1000 mg/kg/day TCB (1.7-fold) (Fig. 3). The activity of the more inducible enzyme ethoxyresorufin *O*-deethylase (a cytochrome *P*-448 type substrate [29]) was greatly increased by β-naphthoflavone pretreatment (13.1-fold). A slight increase of activity was seen in hepatic microsomes from rats fed 300 mg/kg/day TCB, but no such increase occurred in the 1000 mg/kg/day TCB group and thus this finding was not dose related.

β-Naphthoflavone increased the maternal UDP-glucuronyltransferase activity towards both *p*-nitrophenol (a late fetal isoenzyme substrate [30]) and phenolphthalein (a neonatal isoenzyme substrate [30]) (Fig. 4). Pretreatment with TCB increased in vitro glucuronidation rates for both substrates at either the intermediate or high dose.

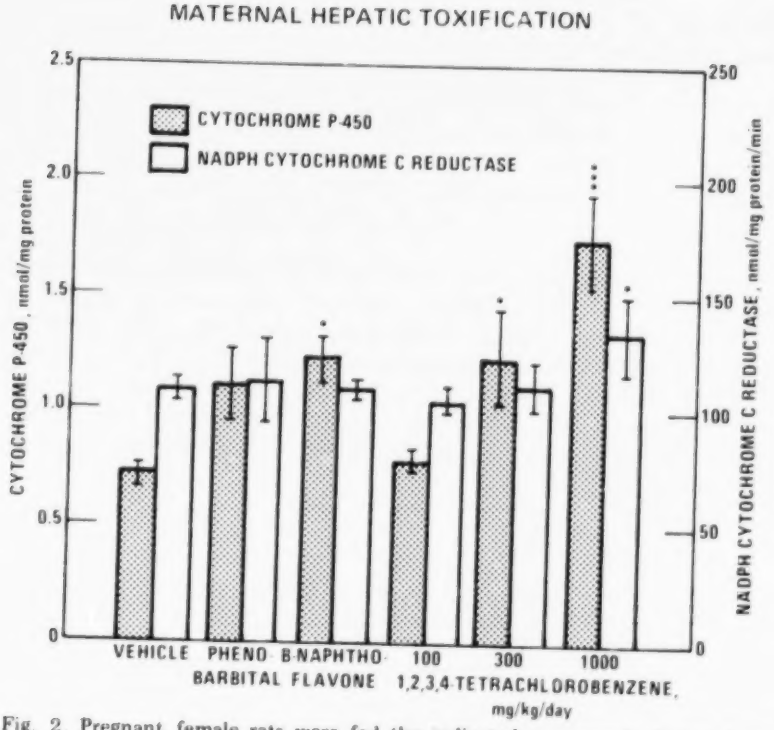

Fig. 2. Pregnant female rats were fed the indicated compounds during days 9—13 of gestation. On day 14 the animals were killed and the hepatic microsomal cytochrome *P*-450 content and NADPH-cytochrome *c* reductase activities determined as described in the Methods. Five to seven animals were used in each group. The data is presented as the mean + S.E.M. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

The second hepatic conjugative enzyme studied was the glutathione-*S*-transferase isoenzymes. In this study only β-naphthoflavone and 1000 mg/kg/day TCB administration increased the enzymatic conjugation of 1,2-dinitro-4-chlorobenzene with glutathione (Fig. 5). Conjugation rates to 1-chloro-2,4-dinitrobenzene or 1,2-epoxy-3-(*p*-nitrophenoxy)propane were not affected by any treatments. In this study approximately 18—20 h elapsed between the last maternal dosing and killing. Even though a 25% decrease in total hepatic glutathione occurred after administration of 1000 mg/kg/day of TCB, no treatment effects were found to be statistically significant. However, when only 5 h elapsed between administration of 300 or 1000 mg/kg of TCB and death, significant decreases in hepatic glutathione (about 45%) were observed.

Gum tragacanth treated controls had unremarkable liver histology. β-Naphthoflavone pretreatment caused minimal hepatocellular hypertrophy and cytoplasmic vacuolization. Phenobarbital administration to pregnant

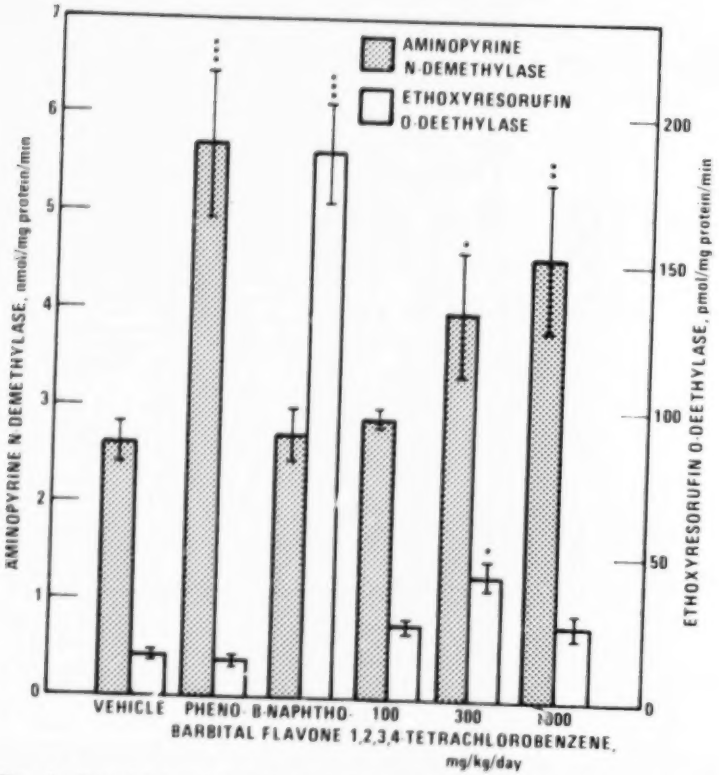

Fig. 3. Day 14 hepatic microsomal aminopyrine *N*-demethylase and ethoxyresorufin *O*-deethylase activities were determined after administration of different chemical pretreatments to rats on days 9–13 of gestation. Data is the mean ± S.E.M. for 5—7 animals. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

rats induced slight to moderate centrolobular hepatocellular hypertrophy. In the TCB treated animals, no hepatic lesions were observed at 100 mg/kg/day. At 300 mg/kg/day 2 of 9 animals had minimal hepatocellular hypertrophy while at 1000 mg/kg/day 9/13 dams showed minimal to moderate hepatocellular hypertrophy.

Administration of 300 mg/kg/day 1,2,3,4-TCB adversely affected yolk sac and embryonic parameters. The treatment means for all 6 yolk sac and embryo parameters were less than the control means. Although the yolk sac protein content was not significantly altered, the day 14 yolk sac diameter was significantly decreased from 9.95 to 8.92 mm. Maternal treatment with TCB did not increase the frequency of embryonic resorptions, lethality or abnormalities. Two of the 4 embryonic growth para-

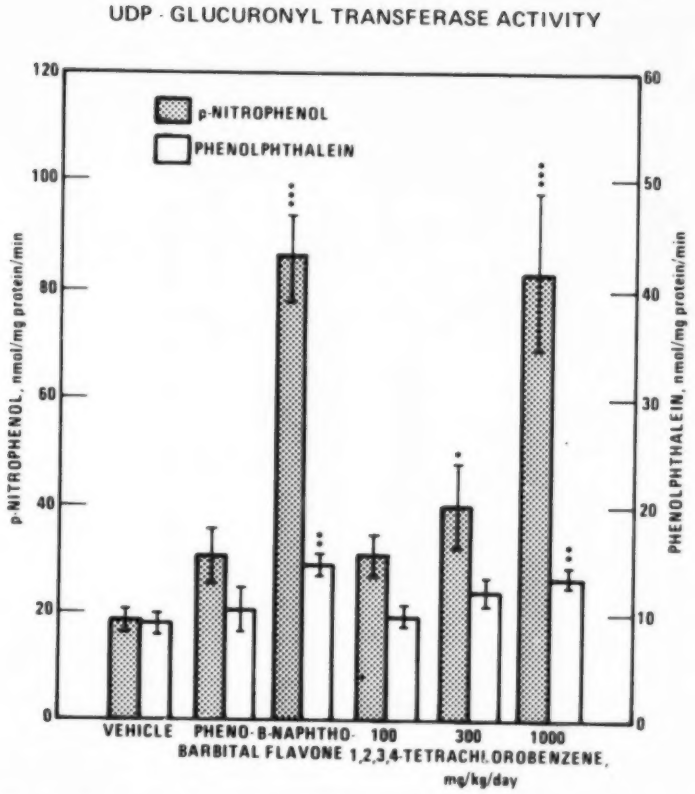

Fig. 4. Pregnant female rats were fed the various compounds indicated during days 9—13 of gestation. On day 14 the animals were killed and the in vitro rates of glucuronidation of *p*-nitrophenol and phenolphthalein determined using 0.2% Triton X-100 activated microsomes. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

meters (head length, crown-rump length) were significantly ($P < 0.05$) reduced by maternal exposure to 300 mg/kg/day TCB (Table I).

DISCUSSION

Hepatic drug metabolism is generally lower in pregnant female rats than in non-pregnant animals [31--33]. The cause of this may be either repression of the various enzymes of drug metabolism [34] or inhibition of the activity of enzymes by endogenous sex steroids [32,35,36].

Administration of phenobarbital caused several anticipated enzyme changes such as the increase in aminopyrine *N*-demethylase and 1,2-dichloro-4-nitrobenzene conjugation with glutathione. Even considering the fact that less responsive pregnant female animals were used, it is somewhat

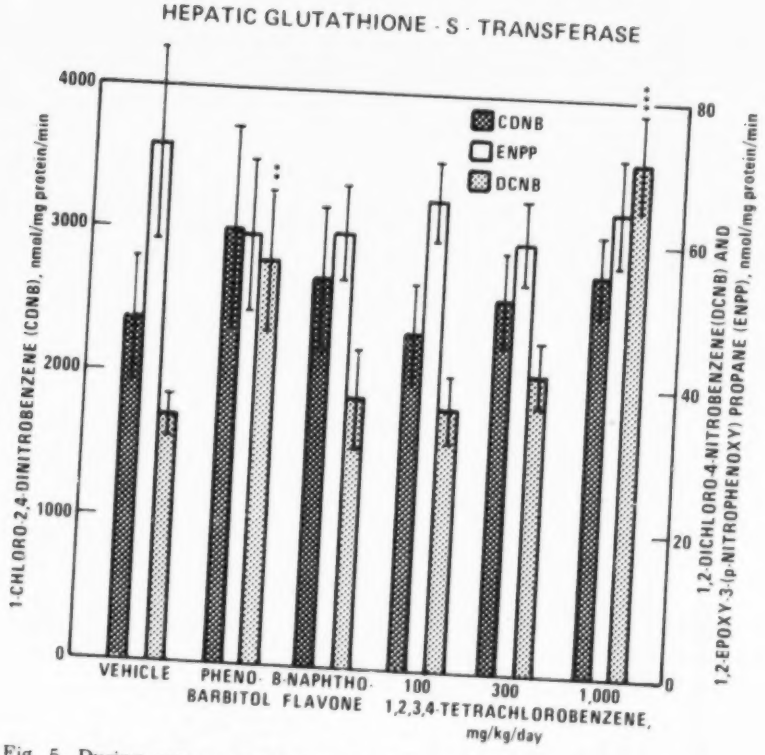

Fig. 5. During organogenesis (days 9–13) pregnant rats were given daily oral doses of the compounds shown. On day 14 of gestation the high speed supernatant (100 000 *g*, 1 h) was used for glutathione *S*-transferase assays. The substrates used were 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB), and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (ENPP). $^{**}P < 0.01$; $^{***}P < 0.001$.

surprising that phenobarbital administration did not increase liver weight, hepatic microsomal protein content, cytochrome *P*-450 level, glucuronyltransferase activity towards phenolphthalein and glutathione *S*-transferase activity towards 1-chloro-2,4-dinitrobenzene. Similar results showing that phenobarbital is a weaker enzyme inducer in pregnant female rats than in non-pregnant animals have been obtained by Guenthner and Mannering [37].

β-Naphthoflavone pretreatment caused the expected battery of responses. Liver weight, hepatic cytochrome *P*-450 content, ethoxyresorufin *O*-deethylase activity and *p*-nitrophenol glucuronidation were all increased. UDP-glucuronidation towards phenolphthalein was increased by 57%, an unexpected finding. Thus the late fetal UDP-glucuronyltransferase isoenzyme activity was increased by prior treatment with β-naphthoflavone and not be phenobarbital, the opposite pattern of that observed by Wishart [38].

TABLE I

EFFECT OF 1,2,3,4-TETRACHLOROBENZENE ON EMBRYONIC GROWTH AND MORPHOGENESIS IN THE RAT

Dams were given daily oral doses of 1.5% gum tragacanth or 300 mg/kg/day TCB on days 9—13 of gestation. Twelve control litters and 15 TCB litters treated were examined. They were killed by ether administration on the morning of day 14 and the uteri examined for implantations, resorptions and embryonic parameters. Two embryos from each female were used in the determination of various embryonic parameters. Quantitative data is expressed as the mean ± S.E.M. for the indicated number of embryonic observations.

	Implantations	Resorptions		Dead Embryos		Embryonic				
		Resorptions/implantations	Affected litters/litters	Affected embryos/embryos	Affected litters/litters	Abnormalities	Head length (mm)	Crown-rump length (mm)	Somites	Protein (μg)
Vehicle	14.2 ± 0.3	9/170	5/12	0/26	0/12	0/26	4.72 ± 0.06 (26)	8.17 ± 0.09 (26)	49.6 ± 0.6 (24)	2670 ± 108.0 (26)
300 mg/kg/day 1,2,3,4-tetrachlorobenzene	12.8 ± 0.7	18/192	9/15	2/30	1/15	1/30	4.33 ± 0.11 (30)	7.64 ± 0.16 (30)	47.9 ± 0.6 (29)	2330 ± 145.0 (29)
Level of significance	NS	NS	NS	NS	NS	NS	$P < 0.05$	$P < 0.05$	NS	NS

The reason for this discrepancy is being investigated. Both ethoxyresorufin *O*-deethylase and *p*-nitrophenol UDP-glucuronyltransferase activity are good markers for the β-naphthoflavone (or aromatic hydrocarbon class) class of inducer. These 2 isoenzymes activities are unaffected by phenobarbital.

At 100 mg/kg/day, TCB caused no effects. At 300 mg/kg/day some phenobarbital (aminopyrine *N*-demethylase and cytochrome *P*-450) and β-naphthoflavone-like (cytochrome *P*-450, ethoxyresorufin *O*-deethylase and UDP-glucuronyltransferase towards *p*-nitrophenol) hepatic enzyme induction occurred.

Ariyoshi et al. fed 250 mg/kg/day of 1,2,3,4-TCB in three daily doses to 110–130 g non-pregnant female Wistar rats. They reported increased liver weight and hepatic microsomal protein, cytochrome *P*-450 content and aminopyrine *N*-demethylase activity [3]. In our studies 300 mg/kg/day of TCB also caused slight though significant embryonic growth retardation in vivo. Further work is required to determine if these embryonic effects are reversible or if 21 day fetuses from dams treated with TCB during organogenesis would be small for gestational age. This compound did not give positive indication of embryonic dysmorphogenesis and all of the TCB-caused embryonic lethality was restricted to a single litter. Thus the data does not suggest that TCB is embryolethal or teratogenic. It is possible that doses of TCB between 300 and 1000 mg/kg would have more pronounced embryonic effects than did the 300 mg/kg dose tested. These results can be compared with previous work from this laboratory on 1,2,4,5-TCB. Of these tetrachlorobenzenes the molecule with 2 vicinal hydrogens (which can thus form epoxides) retarded embryo growth more (albeit a quantitatively small difference) than the molecule without 2 vicinal hydrogens. This is some positive evidence that reactive intermediates (formed in either the maternal or embryonic compartments) can be associated with embryonic developmental problems such as growth retardation. Additional experimental work with 1,2,4-trichlorobenzene is in support of the reactive intermediate theory. Equal molar doses of 1,2,4-trichlorobenzene to pregnant female rats caused more embryonic growth retardation than either the 1,2,4,5- or 1,2,3,4-TCB [39].

Comparison of gestational weight gains and embryonic growth retardation of rats given 1,2,3,4- and 1,2,4,5-TCB showed that significant maternal weight gain depressions and significant growth retardation were not well correlated. 1,2,3,4-TCB caused embryonic growth retardation without a maternal body weight effect. 1,2,4,5-TCB showed the opposite pattern of no embryonic growth retardation in spite of a significant decrease in maternal body weight gain.

At a dose of 1000 mg/kg/day of TCB, 37% maternal lethality was noted, therefore no embryonic growth or morphology studies were done at this dose. This TCB dose greatly affected maternal and liver weights. It also strongly induced total cytochrome *P*-450 levels and several enzyme activities of the phenobarbital (aminopyrine *N*-demethylase and glutathione

S-transferase towards 1,2-dichloro-4-nitrobenzene) and β-naphthoflavone (glucuronidation of *p*-nitrophenol) classes. Although *p*-nitrophenol glucuronidation was equivalently induced by β-naphthoflavone and TCB, TCB did not have much effect on ethoxyresorufin *O*-deethylase activity. Administration of 1,2,3,4-TCB led to hepatic enzyme induction of both the phenobarbital and β-naphthoflavone type. Unlike the previous 1,2,4,5-TCB study [40], with 1,2,3,4-tetrachlorobenzene both conjugative as well as oxidative hepatic enzymes were induced. On a dose basis 1,2,3,4-TCB is the more potent maternal and embryonic toxin of the 2 TCB congeners.

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REFERENCES

1 J.R. Allen, W.A. Hargraves, M.T.S. Hsia and F.S.D. Lin, Comparative toxicology of chlorinated compounds on mammalian species. Pharmacol. Ther., 7 (1979) 513.
2 S.R. Ware and W.L. West, Investigation of selected potential environmental contaminants: Halogenated benzenes final report — Office of toxic substances, EPA (1977).
3 T.K. Ariyoshi, K. Ideguchi, K. Iwasaki and M. Arakai, Relationship between chemical structure and activity. II. Influences of isomers in dichlorobenzene, trichlorobenzene and terachlorobenzene on the activities of drug-metabolizing enzymes. Chem. Pharm. Bull, 23 (4) (1975) 824.
4 J. Kohli, D. Jones and S. Safe, The metabolism of higher chlorinated benzene isomers. Can. J. Biochem., 54 (1976) 203.
5 K.T. Kitchin and M.T. Ebron, Comparison of the maternal hepatic and embryonic effects of 1,2,4,5- and 1,2,3,4-tetrachlorobenzene in the rat. Teratology, 25 (1982) 55A.
6 K.T. Kitchin, B.P. Schmid and M.K. Sanyal, Teratogenicity of cyclophosphamide in a coupled microsomal activating/embryo culture system. Biochem. Pharmacol., 30 (1981) 59.
7 T.A. Connors, P.J. Cox, P.B. Farmer, A.B. Foster and M. Jarman, Some studies of active intermediates formed in microsomal metabolism. Biochem. Pharmacol., 23 (1974) 115.
8 L.G. Hart, R.H. Adamson, R.L. Dixion and J.R. Fouts, Stimulation of hepatic microsomal drug metabolism in the newborn and fetal rabbit. J. Pharmacol. Exp. Ther., 137 (1962) 103.
9 K.T. Kitchin, Enhanced aryl hydrocarbon hydroxylase activity after interaction between solubilized cytochrome *P*-448 and microsomes low in endogenous cytochrome *P*-450. Biochem. Pharmacol., 29 (1980) 1261.
10 S.M. Galloway, P.E. Perry, J. Menesser, D.W. Nebert and R.A. Pedersen. Cultured mouse embryos metabolize benzo(a)pyrene during early gestation: Genetic differences detectable by sister chromatid exchange. Proc. Natl. Acad. Sci. USA, 77 (1980) 3524.
11 Filler and K.J. Lew, Developmental onset of mixed function oxidase activity in preimplantation mouse embryos. Proc. Natl. Acad. Sci. USA, 78 (1981) 6991.

12 Poland and E. Glover, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: segration of toxicity with the Ah locus. Mol. Pharmacol., 17 (1980) 86.
13 T. Sawahata, J.R. Olson and R.A. Neal, Identification of metabolites of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) formed on incubation with isolated rat hepatocytes. Biochem. Biophys. Res. Commun., 105 (1982) 341.
14 K.T. Kitchin and J.S. Woods, 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) effects on hepatic microsomal cytochrome *P*-448 mediated enzyme activities. Tox. Appl. Pharmacol., 47 (1979) 537.
15 D.A.T. New, Whole embryo culture and the study of mammalian embryos during organogenesis. Biol. Rev., 53 (1978) 81.
16 K.T. Kitchin and J.S. Woods, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) induction of aryl hydrocarbon hydroxylase in female rat liver. Evidence for de novo synthesis of cytochrome *P*-448. Mol. Pharmacol., 14 (1978) 890.
17 T. Omura and R. Sato, The carbon monoxide-binding pigment of liver microsomes. J. Biol. Chem., 239 (1964) 2370.
18 B.N. LaDu, H.G. Mandel and E.L. Way, Fundamentals of drug metabolism and disposition, Williams & Wilkins, Baltimore, 1971, p. 575.
19 T. Nash, The colorometric estimation of formaldehyde by means of the Hantzsch reaction. Biochem. J., 55 (1953) 416.
20 G. Lucier, O. McDaniel, P. Brubaker and R. Klein, Effects of methylmercury hydroxide on rat liver microsomal enzymes. Chem.-Biol. Interact., 4 (1971) 265.
21 M.D. Burke and R.T. Mayer, Ethoxyresorufin: Direct fluorometric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metab. Dispos., 2 (1974) 583.
22 W.H. Habig, M.J. Pabst and W.B. Jakoby, Glutathione *S*-Transferases, the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249 (1974) 7130.
23 J. Sedlak and R.H. Lindsay, Estimation of total, protein-bound, and nonprotein sulfhydryl group in tissues with Ellman's reagent. Anal. Biochem., 25 (1968) 192.
24 G.J. Mulder, The effect of phenobarbital on the submicrosomal distribution of uridine diphosphate glucuronyltransferase from rat liver. Biochem. J., 117 (1970) 319.
25 A.G. Gornall, C.J. Bardawill and M.M. David, Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177 (1949) 751.
26 A. Goldstein, Biostatistics, MacMillan Co., New York, 1964, p. 63.
27 D.A. Williams, A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics, 27 (1971) 103.
28 D.A. Haugen, M.J. Coon and D.W. Nebert, Induction of multiple forms of mouse liver cytochrome *P*-450. J. Biol. Chem., 251 (1976) 1817.
29 M.D. Burke and R.T. Mayer, Inherent specificities of purified cytochromes *P*-450 and *P*-448 toward biphenyl hydroxylation and ethoxyresorufin deethylation. Drug Metab. Dispos., 3 (1975) 245.
30 G.J. Wishart, M.T. Campbell, G.J. Dutton and A. Aitio, Functional heterogeneity of UDP-glucuronyltransferase, in Conjugation Reactions in Drug Biotransformation, Elsevier/North-Holland Biomedical Press, 1978, p. 179.
31 J.E. King and R.F. Becker, Sex differences in the response of rats to pentobarbital sodium. Am. J. Obstet. Gynecol., 86 (1963) 856.
32 G. Feuer and R. Kardish, Hormonal regulation of drug metabolism during pregnancy. Int. J. Clin. Pharmacol., 11 (1975) 366.
33 G. Feuer, Action of pregnancy and various progesterones on hepatic microsomal activities. Drug Metab. Rev., 9 (1979) 147.
34 A.M. Guarino, T.E. Gram, D.H. Schroeder, J.B. Call and J.R. Gillette, Alterations in kinetic constants for hepatic microsomal aniline hydroxylase and ethylmorphine *N*-demethylase associated with pregnancy in rats. J. Pharmacol. Exp. Ther., 168 (1969) 224.
35 M.R. Juchau and J.R. Fouts, Effects of norethynodrel and progesterone on hepatic

microsomal drug-metabolizing enzyme system. Biochem. Pharmacol., 15 (1966) 891.
36 T.R. Tephly and G.J. Mannering, Inhibition of microsomal drug metabolism by steroid hormones. The Pharmacologist, 6 (1964) 186.
37 T.M. Guenthner and G.J. Mannering, Induction of hepatic mono-oxygenase systems of pregnant rats with phenobarbital and 3-methylcholanthrene. Biochem. Pharmacol., 26 (1977) 577.
38 G.J. Wishart, Demonstration of functional heterogeneity of hepatic uridine diphosphate glucuronyltransferase activites after administration of 3-methylcholanthrene and phenobarbital to rats. Biochem. J., 174 (1978) 671.
39 K.T. Kitchin and M.T. Ebron, Maternal hepatic effect of 1,2,4-trichlorobenzene in the rat. Environ. Res., (in press).
40 K.T. Kitchin, Hepatic effects of 1,2,4,5-tetrachlorobenzene in the pregnant rat. The Toxicologist, 2 (1982) 506.